

Modification of Wheat Straw in a High-Shear Mixer

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Accepted for Publication October 12, 1983

Wheat straw (WS) was treated in a pilot-scale continuous mixer to disrupt the lignin-hemicellulose-cellulose (LHC) complex. An efficient and practical method was desired to remove lignin and hemicellulose (pentosans) rapidly and efficiently from the lignocellulose complex and to make the cellulose accessible to enzymatic hydrolysis. Milled WS in the presence of various chemicals in aqueous solutions was extruded from the mixer under several processing conditions. Chemicals used were sodium hydroxide (NaOH), sodium sulfide (Na_2S), anthraquinone (AQ), anthrahydroquinone (AHQ), hexamethylenediamine (HMDA), hexamethylenetetramine (HMTA), hydrogen peroxide (H_2O_2), and ferrous ammonium sulfate (FAS), which were used alone and in selected combinations. Concomitantly, WS was treated in laboratory batches using similar reaction conditions, except for mixing and shearing. In extrusion treatments of WS at 20% concentration at 97°C for 5.5 min with NaOH (15.7%, dry WS basis), NaOH (15.7%) + AHQ (0.3%), and NaOH (12.7%) + Na_2S (5.0%), 64–72% of the WS lignin and 36–43% of the pentosans were removed from aqueously washed extrudates (residues). This compares with 46–56% and 23–27%, respectively, for batch treatments. AHQ and Na_2S enhanced delignification. Cellulase treatment of the residues, which contained about 99% of the WS cellulose, converted 90–92% of the cellulose to glucose compared with 61–69% for the batch pretreatments. Treatments of WS with amines and H_2O_2 (alone or combined with NaOH) were less effective for LHC disruption. In all instances the relatively high-shear extrusion treatments were superior to the laboratory-batch treatments.

INTRODUCTION

Interest in lignocellulosics as renewable sources of energy has developed and broadened rapidly. A major requirement for converting these raw materials to fermentable sugars is to develop more efficient pretreatments to disrupt the lignin-hemicellulose-cellulose (LHC) complex. Treatments that remove lignin and hemicellulose

from the LHC complex without destroying fiber structure are inadequate for obtaining a high degree of cellulose accessibility to enzymatic hydrolysis. Such accessibility can be achieved by exposing a large surface area or destroying crystallinity of the cellulose. A variety of methods have been studied for separating LHC components and rendering the substrates readily hydrolyzable. These include solvation,^{1,2} fine grinding,³ microfibrillation,⁴ irradiation,⁵ ultrasonics,⁶ explosive depressurization,⁷ freeze explosion,⁸ chemical derivatization,⁹ and wood-pulping techniques.^{10,11}

Extrusion cooking is a rapidly advancing technology for processing food, feed, and plastics.^{12–14} Little basic information is available on the effects of processing such materials under high-shear extrusion conditions.¹² However, the extrusion process *per se* is technologically simple¹³ and offers an efficient technique for effecting physiochemical changes.^{12–14} Consequently, there is increasing interest in extrusion treatment (primarily acid hydrolysis) of biomass.^{13–19}

Previously, our Center investigated several physical and chemical pretreatments of wheat straw (WS) to study their effect on composition, accessibility, and fermentability of the carbohydrate constituents.^{20,21} We now report on WS treated in a pilot-scale, extrusion-type mixer to determine the influence of chemicals and high shear on composition of the extrudates, and on accessibility of cellulosic residue to enzymatic hydrolysis. The mixer has an interrupted-flight screw that rotates and reciprocates past fixed kneading teeth inside a barrel. A high degree of mixing can be achieved rapidly as reported in xanthation²² and enzymatic liquefaction²³ studies of starch. Our report discusses the effect of selected chemicals in aqueous solution on WS extruded under various conditions. Residues from the extrudate were analyzed quantitatively for lignin, pentosans, and cellulose and treated with cellulase to determine accessibility of the carbohydrates. Although the present report does not discuss potential end uses of the lignin and pentosan fractions, the value of these components from extrusion processing is now being studied with emphasis on total biomass utilization.

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

EXPERIMENTAL

Wheat Straw (WS)

Straw from Soft winter wheat (*Triticum aestivum*, variety Arthur) containing 7% moisture was prepared in a Thomas-Wiley mill (model ED-5) equipped with a sieve having 2-mm-diameter openings.

Chemicals

Chemicals were reagent grade. Hydrogen peroxide (H_2O_2) was refrigerated at 30% concentration and diluted at time of use. Ferrous ammonium sulfate (FAS) was the hexahydrate. Anthrahydroquinone (AHQ) was prepared from anthraquinone (AQ) as follows: 3 g AQ in 30 mL of 95% ethanol and 7.5 g of sodium dithionite in 25 mL of water were refluxed 15 min, cooled to 25°C, filtered under vacuum, and washed with 25 mL of H_2O . AHQ was dissolved in concentrated NaOH to form the disodium salt and diluted at time of use.

Cellulase

Cellulase was Takamine Brand, TV Concentrate, product number 4522000 (Enzyme Products, Miles Laboratories, Inc., Elkhart, IN). The product is a multienzyme complex obtained by fermentation of *Trichoderma viride*, which hydrolyzes β -D-1,4-glucosidic bonds of cellulose. The product is sold as an unstandardized concentrate, typically assaying 10,000–20,000 viscometric cellulase units per gram.

Continuous Mixer

The mixer was a model 50 Ko-Kneader, manufactured by Baker Perkins, Inc., Chemical Processing Equipment.^{22–24} It has a variable speed (19–93 rpm), 1.95-in. diameter, interrupted-flight screw that reciprocates 0.375 in. each revolution past 62 stationary cylindrical pins situated in 3 rows 120° apart inside a 2.0-in.-diam \times 18-in. long barrel. The end of the screw was equipped with a solid cylindrical die, which gave an annular discharge opening of 0.37 in.² All parts in contact with WS were stainless steel. The mixer was equipped with a variable-speed screw feeder, two high-pressure diaphragm pumps, and thermocouple wires at the tips of cylindrical pins located near the mouth, middle, and extrusion end of the barrel. Thermocouple wires were attached to a temperature recorder. Temperatures were controlled by circulating steam- H_2O through the hollow-shaft screw and through two jackets surrounding the barrel.

Extrusion Treatment Procedure

This procedure is exemplified as follows: The screw speed was adjusted to 35 rpm and the barrel temperature to 98°C. Milled WS (7% moisture) was fed into the

mouth of the barrel by the variable-speed screw feeder at 10.8 g (10.0 g d.b.)/min, and 39.3 mL NaOH (4.0% w/v)/min was metered by a high-pressure diaphragm pump into the barrel through a port directly below the mouth. These conditions produced extrudate that had 15.7% NaOH (dry WS basis), 20% WS concentration (4/1, water/straw), temperatures of $97 \pm 1^\circ\text{C}$ (9–17 in. downstream), and a residence period of 5.6 ± 1 min. Residence time was determined by injecting dye into the mouth of the barrel. After steady-state operation of 30 min, extrudate was collected 10 min in a bucket containing 1000 g of crushed ice while constantly stirring by hand. When more than one chemical was used, they were combined as one solution, except for H_2O_2 + FAS since FAS decomposes H_2O_2 . In this case, H_2O_2 was added as described for NaOH, and FAS solution was pumped into the barrel through a hollow kneading pin, 1 in. downstream from the H_2O_2 inlet.

Extrudate Sample Handling

Each sample of extrudate with the added 1000 g of ice was diluted with 2000 mL of H_2O , filtered through a 37- μm mesh sieve, and washed five times with 1000-mL portions of H_2O (30°C). Both the residue and liquor solids were freeze dried.

Laboratory Batch Procedure

Laboratory-batch treatment of WS is exemplified as follows: To 10.8 g of WS (10.0 g d.b.) in a 250-mL beaker, held in a boiling water bath, was added 39.3 mL of nearly boiling 4.0% NaOH. The mixture was heated 5.5 min while stirring as well as possible with a 0.25-in.-diameter stainless steel rod. After the beaker was removed from the bath, the contents were immediately cooled with ice to 10°C, diluted with H_2O , and washed exhaustively (37- μm sieve).

Analyses

Analyses were (1) pentosans by TAPPI Standard Method T223 m, (2) lignin by ultraviolet spectroscopy,²⁵ (3) cellulose by a monoethanolamine (MEA) method,²⁶ and (4) ash by sample ignition at $600 \pm 25^\circ\text{C}$. Cellulose was analyzed for pentosans and ash and reported as MEA cellulose on a dry, ash-free, and pentosan-free basis.

Cellulase Hydrolysis and Sugar Analysis

Cellulase treatment was similar to that previously reported.²⁷ Treatment was done in duplicate on 250-mg samples of residue from the extrudate in 50-mL Erlenmeyer flasks in a water bath shaker (160 strokes/min) at 40°C, 2.5% WS concentration, and pH 4.0 (McIlvaine's standard buffer solution) for 24 h. Cellulase dosage was 100 mg TV concentrate/250 mg residue.

RESULTS AND DISCUSSION

Extrusion Treatments

Table I shows effect of various chemicals used in extrusion treatments on yield of residue and removal of lignin, pentosans, and cellulose from WS. Extrusion conditions were 10 g WS (d.b.)/min, 20% WS concentration, 97°C, 35 rpm (screw speed), and 5.5 min (WS residence time). Chemicals are the type used in alkaline pulping and bleaching to remove lignin and noncellulose fractions from wood. With 15.7% NaOH (dry WS basis), which is a concentration used in alkaline pulping,¹¹ yield of residue was 65%. Correspondingly, 64% of the WS lignin and 43% of the pentosans were removed (see calculations in Table I, footnotes c and d).

Much interest has been shown in AQ as a delignification catalyst in alkaline pulping.^{28,29} The most accepted mechanism is that AQ is reduced by carbohydrates to AHQ or its radical anion, which is reoxidized to AQ by lignin in a series of oxidation-reduction reactions.²⁸ An attempt to use AQ in our process was impractical because it is insoluble in NaOH solutions, even on heating. Therefore, AQ was chemically reduced to AHQ (see Experimental section) and used as its alkali-soluble disodium salt. Use of 0.3% AHQ + 15.7% NaOH gave a 63% residue yield while removing 70% of the WS lignin and 40% of the pentosans.

Na₂S and NaOH combined are used in kraft wood pulping. Water and Na₂S form NaOH and NaSH in equilibrium ($\text{Na}_2\text{S} + \text{H}_2\text{O} \rightleftharpoons \text{NaOH} + \text{NaSH}$). As NaOH is consumed, the equilibrium shifts to the right and NaSH promotes delignification. In our extrusion process 12.7% NaOH + 5.0% Na₂S was used. This corresponds to a to-

tal effective alkali¹¹ of 11.8% (12.2% for 15.7% NaOH addition) and a sulfidity¹¹ of 28.8%, which are levels used in kraft pulping.¹¹ This treatment removed 72% lignin, 36% pentosans, and 3% cellulose; yield was 64%.

Amine compounds are receiving renewed interest for removing lignin and for developing sulfur-free pulping systems. Either ethylenediamine (EDA) or hexamethylenediamine (HMDA) combined with NaOH is effective in high temperature-pressure digester systems.²⁹ In our work 12.4% HMDA and 8.5% NaOH were used alone and in combination. Also, 7.5% hexamethylenetetramine (HMTA) + 8.5% NaOH was used. These levels represent 2.12×10^{-3} mol of each active group ($-\text{NH}_2$ and OH^-)/g WS. On the basis of active group concentration, HMDA was about as effective as NaOH for removing lignin and pentosans. However, a cumulative effect for HMDA + NaOH did not occur. Results were similar for HMTA + NaOH.

Hydrogen peroxide (H₂O₂) in alkaline pulping systems is well known for its bleaching effect without removal of lignin.³⁰ It is generally believed that bleaching is by the HO₂⁻ anion. It is also reported that metal salts + H₂O₂ bleach cotton, linen, and pulp.^{30,31} Ferrous ions + H₂O₂ (Fenton's reagent), for example, form hydroxyl radicals ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{HO}\cdot + \text{HO}^-$), which can effect delignification.³⁰ In our study the peroxide treatments (4.0% NaOH + 2.0% H₂O₂, 4.0% NaOH + 2.0% H₂O₂ + 1.08% Fe²⁺, and 2.0% H₂O₂ + 1.08% Fe²⁺) were not very effective in removing WS components (1–8% lignin and 5–18% pentosans) during such short treatment periods (5.5 min). However, H₂O₂ treatments with Fe²⁺ were slightly more effective than without Fe²⁺.

Table I. Effect of extrusion treatments on WS.^a

Additive		WS components removed (% ^d)			
Type	(% ^b)	Residue yield (% ^c)	Lignin	Pentosans	Cellulose
Water only		95	4	0	<1
NaOH	15.7	65,65,65	65,66,61	45,41,45	<1
NaOH + AHQ ^e	15.7 + 0.3	63,63	70,72	40,38	<1
NaOH + Na ₂ S	12.7 + 5.0	64	72	36	3
NaOH	8.5	85	35	5	<1
NaOH + HMDA ^e	8.5 + 12.4	76	48	17	1
HMDA	12.4	80	30	16	6
NaOH + HMTA ^e	8.5 + 7.5	86	30	5	<1
NaOH + H ₂ O ₂	4.0 + 2.0	89	1	5	8
NaOH + H ₂ O ₂ + Fe ^f	4.0 + 2.0	88	6	18	5.0
H ₂ O ₂ + Fe ^f	2.0	88	8	16	6

^aExtrusion treatments: 10 g WS (d.b.)/min, 20% WS concentration, 97°C, 35-rpm screw speed (5.5-min residence). NaOH and NaOH + AHQ runs were in triplicate and duplicate, respectively.

^bPercent, dry WS basis.

^cPercent = g residue ÷ (g residue + g liquor solids) × 100. All calculations on a dry, ash-free, sample weight basis.

^dPercent = $100 - [(\% \text{ component in residue} \times \% \text{ residue yield}) \div \% \text{ component in untreated WS}]$. Untreated WS contained 16.0% lignin, 32.0% pentosans, and 34.0% MEA cellulose on a dry, ash-free, sample weight basis.

^eAHQ = anthrahydroquinone; HMDA = hexamethylenediamine; HMTA = hexamethylenetetramine.

^fFe = ferrous iron. 7.5% Fe (NH₄)₂ (SO₄)₂ · 6H₂O (equal 1.08% Fe) on dry WS basis.

Extrusion Variables

Table II shows the effect of treatment variables on WS treated with 15.7% NaOH. The reference treatment in Table II refers to conditions described for Table I (footnote a). Temperature had more effect on removing pentosans than it did on lignin removal. The amounts of pentosans removed from WS with temperatures of 97 (reference treatment), 70, and 50°C were 43, 30, and 22%,

Table II. Effect on extrusion variables in alkali treatment of WS.^a

Variable	Residue yield (% ^b)	Components removed (% ^c)	
		Lignin	Pentosans
Reference treatment ^d	65	64	43
Temperature (°C)			
50	82	53	22
70	77	51	30
Screw speed (rpm)			
24	68	56	31
65	65	64	37
93	64	60	42
Extrusion rate (g WS/min)			
20 (65 rpm)	65	63	41
30 (95 rpm)	65	63	39
WS concentration (% ^e)			
12	68	59	34
33	67	60	32

^aAlkali = 15.7% NaOH, dry WS basis.

^bSee Table I, footnote c.

^cSee Table I, footnote d.

^dSame conditions as shown in Table I, footnote a. Values are averages of replicate run shown in Table I.

^eWater/water + dry WS (w/w) basis.

respectively, compared with 64, 51, and 53% of lignin. Correspondingly, residue yields were 65, 77, and 82%.

Incremental increases in screw speed from 24 to 93 rpm resulted in moderate increases in removal of lignin (56% at 24 rpm and 64% at 93 rpm) and pentosans (31 and 43%). However, the effect of shear rate, which increases with screw rpm, appears important when considering that WS residence time was 6.5 min at 24 rpm compared with 3.1 min at 93 rpm. Changes in WS feed rates from 10 g/min at 35 rpm (reference treatment) to 20 g/min at 65 rpm (4.0-min residence) and to 30 g/min at 93 rpm (3.25 min) did not significantly alter results. Essentially, plug flow processing conditions existed with feed rates of 10, 20, and 30 g/min at 24–35 rpm, 65 rpm, and 95 rpm, respectively. With 10 g/min at 65 and 93–95 rpm, straw was processed under starved feed conditions. WS concentration of 20% (reference treatment) was only slightly better than either 12 or 33% for removing lignin (64% vs. 59–60%) and pentosans (43% vs. 39–41%). An advantage of high-solids treatments would be that substantial energy savings could be realized, using a minimum of heated water.

Process power was monitored by a wattmeter connected to the drive motor of the mixer. Applied specific energy values for each run were calculated by dividing net horsepower (HP) by pound of WS (dry basis) processed per hour. These values ranged from only 0.051 to 0.061 HP h/lb for the various treatments discussed in Table I and for the temperature and concentration variables in Table II. Specific energy values were 0.051, 0.061, 0.081, and 0.100 for 24, 35, 65, and 95 rpm, respectively, using a processing rate of 10 g WS/min. Power consumption did not increase significantly by increasing the processing rate from 10 to 30 g WS/min.

Table III. Cellulase treatment of extruded WS residues.^a

Additive		Cellulase-treated residue		
Type	(% ^b)	Total WS solubles (%)	Cellulose to glucose (% ^c)	Pentosan to xylose (% ^c)
None ^d		5	20	10
Water only		12	29	9
NaOH	15.7	76	90	43
NaOH + AHQ ^e	15.7 + 0.3	75	90	40
NaOH + Na ₂ S	12.7 + 5.0	78	92	44
NaOH	8.5	59	75	39
NaOH + HMDA ^e	8.5 + 12.4	68	71	27
HMDA	12.4	29	32	10
NaOH + HMTA ^e	8.5 + 7.5	56	56	23
NaOH + H ₂ O ₂	4.0 + 2.0	12	23	11
NaOH + H ₂ O ₂ + Fe ^e	4.0 + 2.0	15	31	12

^aSame conditions as shown in Table I, footnote a. See Experimental section for cellulase treatment.

^bPercent, dry WS basis.

^cPercent of cellulose converted to glucose was calculated as follows: g of glucose produced ÷ [(% MEA cellulose of residue ÷ 100) × g of sample × (180 ÷ 162)] × 100. Percent pentosan converted to xylose = g xylose ÷ [(% pentosans ÷ 100) × 0.9 × g of sample (150 ÷ 132)] × 100. Ratio of xylose to total pentosans in WS is 0.9.

^dMilled straw received no treatment other than cellulase.

^eSee Table I, footnotes e and f.

Table IV. Effect of batch chemical treatments on WS.^a

Laboratory-batch treatment additive		Residue yield (%)	Components removed (%)		Cellulase-treated residue
Type	(%)		Lignin	Pentosans	cellulose to glucose (%)
Water only		93	4	3	17
NaOH	15.7	73	46	23	61
NaOH + AHQ	15.7 + 0.3	69	55	27	69
NaOH + Na ₂ S	12.7 + 5.0	70	56	24	67

^aSee Experimental section for treatment detail. See Table I, footnotes b-e for tabular definitions.

Cellulase Hydrolysis

Effect of chemical extrusion treatments on accessibility of cellulosic residue to cellulase hydrolysis (see Experimental section) is shown in Table III. In general, accessibility was highly related to amounts of lignin and pentosans removed from WS. Cellulose crystallinity of the treated WS was not examined. After treating WS with NaOH (15.7%), NaOH (15.7%) + AHQ (0.3%), and NaOH (12.7%) + Na₂S (5.0%), cellulase converted 76–78% of the residues to solubles, including 90–92% of the cellulose to glucose (see calculation in footnote c). Cellulose of milled untreated WS and of WS extruded with only water (97°C) were 20 and 29% accessible, respectively. Cellulase also produced significant amounts of xylose, representing 43–44% of the pentosans in the residues. About 90% of the pentosans in the untreated WS was composed of xylose units.

Treatments of WS with 8.5% NaOH allowed cellulase to convert 75% of the cellulose to glucose. Inexplicably, combinations of NaOH (8.5%) with either HMDA (12.4%) or HMTA (7.5%) and with HMDA alone were less effective to various degrees than NaOH alone. The H₂O₂ treatments were not effective pretreatments for obtaining accessibility—possibly because of the short treatment period (5.5 min).

Batch Chemical Treatments

WS in 10-g batches was treated at 20% concentration and 95–97°C for 5.5 min with the same levels of chemicals used in extrusion treatment (see Experimental section on batch treatment). Table IV shows that NaOH, NaOH + AHQ, and NaOH + Na₂S removed 46–56% lignin and 23–27% pentosans (residue yields, 69–73%). This compares with 64–72% lignin and 36–43% pentosans, respectively, for extrusion treatments (Table I). Similarly, cellulose of residues from batch treatments was less accessible to cellulase hydrolysis (61–69% cellulose to glucose) than were residues from extrusion treatments (90–92% cellulose to glucose). The greater disruption of the LHC complex by extrusion treatments (compared with batch) using a variety of experimental conditions demonstrates the importance of simultaneous

physical and chemical interaction under high shear and provides the basis for extended study of lignocellulosics under considerably higher shear rate.

The authors thank F. F. Long, D. M. Palmer, and D. W. Ehmke for their technical assistance.

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